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14. ABSTRACT

The humoral response of a cancer patient may allow earlier detection of cancer than current methods allow. If so, the serum autoantibody repertoire from cancer patients might be exploited for autoantibody profiling, and aid in the serological diagnosis of cancer. In this final report, we report on the development of a whole proteome native antigen microarray for human breast cancer. Using this platform and sera from stage 1 and 2 invasive ductal carcinoma of the breast as well as normal controls, we identified antigen containing fractions that were significantly differentially reactive with the cancer sera (p≤0.05). Receiver operator characteristics curves were plotted for the top 5 reactive fractions and the area under the curve (AUC) was calculated. Our findings showed that when combined, the 5 reactive fractions have an AUC of 0.89 for stage 1 breast cancers versus normal controls, and an AUC of 0.82 for stage 2 breast cancers versus normal controls. Our results suggest that our platform can identify specific immune response signatures that might serve as biomarkers.

15. SUBJECT TERMS
Whole proteome native antigen microarray; biomarkers; autoantibody; breast cancer

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INTRODUCTION:

Cancer sera contain antibodies that react with a unique group of autologous cellular antigens called tumor-associated antigens (TAAs). Proteins not present in normal cells may elicit a host immune response, which affords a dramatic amplification of signal in the form of antibodies relative to the amount of the corresponding antigen. In addition, the humoral response may allow earlier detection of cancer than current methods would allow. If so, the serum autoantibody repertoire from cancer patients might be exploited for autoantibody profiling, and potentially aid in the serological diagnosis of cancer. To date, however, studies of antigen-autoantibody reactivity using protein microarrays have relied on recombinant proteins or synthetic peptides as arrayed features. This current approach may fail to accurately detect autoantibody binding due to the lack of proper post-translational modifications and antigen folding. In this final report, we report on the development of a whole proteome native antigen microarray platform for autoantibody profiling of human breast cancer, and demonstrate its utility in identifying relevant autoantibody signatures for early stage breast cancer.

BODY:

Even with the use of expression systems such as the baculovirus expression system, which renders protein modifications observed with mammalian cells, such post-translational modifications (PTMs) remain dependent of the expression systems that are used and may not reflect the unique modifications that are associated with a human disease. It is also important to note that several studies have shown that PTMs on proteins serve as stimuli or epitopes for autoantibody reactivity (1-3). Thus, the objectives of our research are to: 1) develop a whole proteome native antigen microarray platform that can be used to monitor humoral immune responses, and 2) test the hypothesis that global autoantibody profiling might identify relevant disease signatures as potential biomarkers.

In brief, we developed a whole proteome native fractionation microarray platform for antigen-autoantibody profiling by separating well-characterized tumor cell lysates into defined antigen fractions based on the antigen's individual chemistry. This was accomplished by using a 2-D liquid chromatography fractionation strategy, where the 1st dimension is separation by isoelectric points and the 2nd dimension is separation by hydrophobicity. This strategy gave us over 1,000 fractions with each fraction containing between 1 and 10 different proteins. Following the 2nd dimension, the fractions containing the tumor antigens were arrayed onto nitrocellulose coated microscope slides (see figure 1, below). These spotted fractions contained proteins in their natural state and, as such, contained relevant post-translational modifications and alterations that are specific to the disease, i.e., cancer, thereby allowing these arrays to detect autoantibody reactivity to disease-related epitopes.

Figure 1: This figure is a schematic showing that the whole proteome from a tumor is fractionated by isoelectric points and hydrophobicity using 2-D liquid chromatography. The different fractions are then printed onto nitrocellulose coated microscope slides, and incubated with labeled serum antibodies. After detection, the informative fractions and their associated antigens are identified using mass spectrometry. Subsequent validation can be performed with other techniques such as ELISA and/or tissue microarrays.
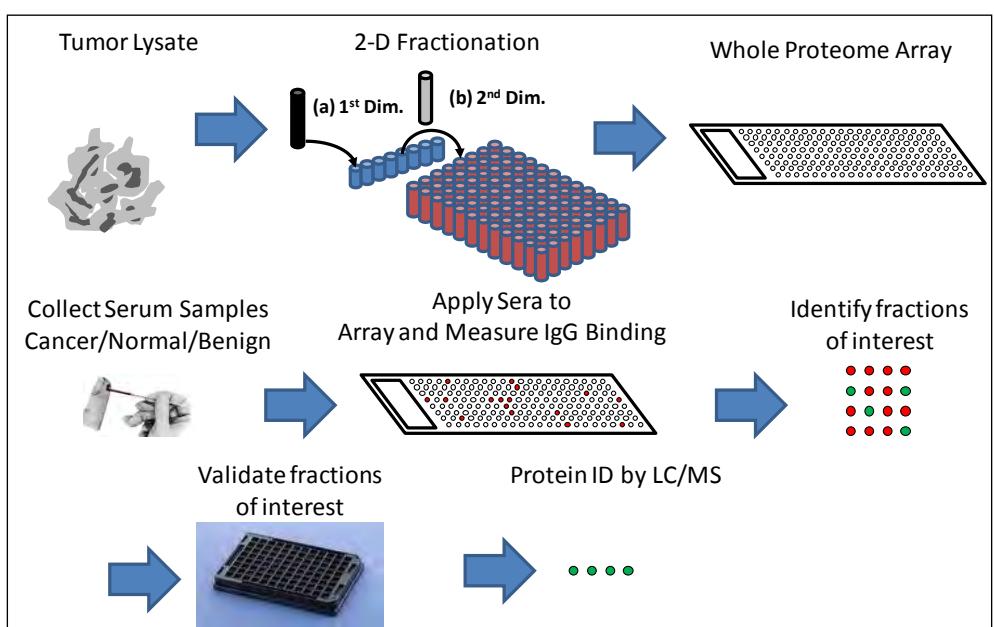
To test the utility of the platform, we initially tested our platform on sera from patients who underwent tumor vaccination with autologous tumor cells engineered to express granulocyte macrophage-colony stimulating factor (GM-CSF). Our assumption is that there should be humoral responses to the cancer cells following vaccination. With IRB approval, sera were obtained from Karen Anderson, M.D., Ph.D., at the Dana-Farber Cancer Institute where she had completed a clinical vaccine trial with patients with late stage breast cancer. Pre- and post-vaccination sera were used. Pre- and post-vaccination IgGs from the same patient were isolated and labeled with fluorescent dyes, and their reactivity to specific fractions on the microarray was compared.

Figure 2: This image (right) demonstrates the reactivity of the IgGs on the whole proteome microarray. Pre-vaccination IgG was labeled with a red fluorescing Cy5 dye, and the post-vaccination IgG from the same patient was labeled with a green fluorescing Cy3 dye. Note that there was little reactivity from the pre-vaccination IgGs, but numerous post-vaccination reactive spots are seen.   When the patients who underwent tumor vaccination were collectively compared (n=7), 4 distinct fractions (spots) were found to have increased responses in over 50% of the vaccinated patients. Some of the proteins in these fractions were then identified using mass spectrometry (Table 1 below).
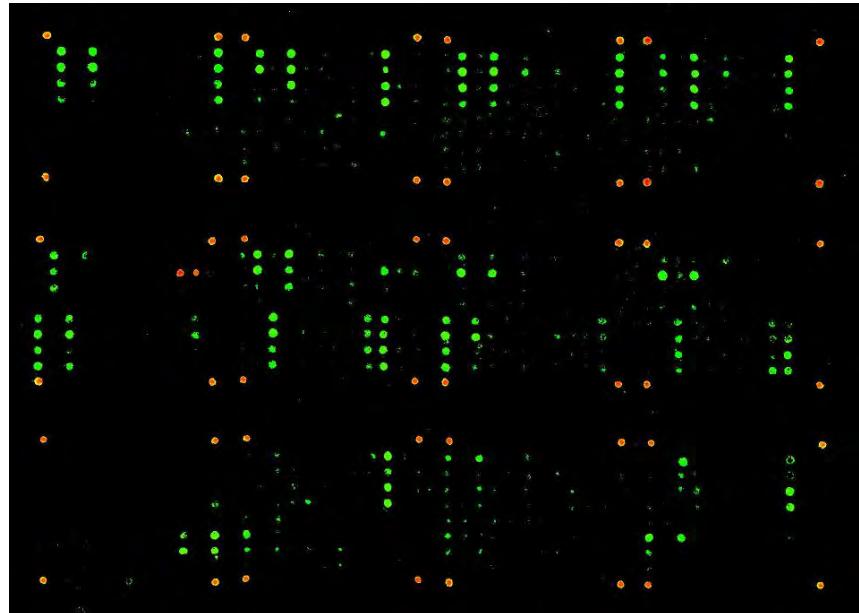
Table 1: Proteins identified by mass spectrometry in the 4 distinct fractions where over 50% of patients were found to have an increased antibody response following post-vaccination with autologous tumor cells.

Protein	Relation to Breast Cancer
Mammaglobin-B	A breast cancer diagnostic marker; highly similar to mammaglobin-A which is over expressed in 23% of primary breast cancer tumors; limited to the epithelial tissue of the mammary glands
Lactotransferrin	Iron transporting glycoprotein with serine protease activity, both of which contribute to its antimicrobial properties; thought to inhibit tumor growth by halting the cell cycle at the G1 to S checkpoint
MUC5B	O-glycoprotein up-regulated in epithelial breast cancer tissue; absent from normal tissue
HSP70	Chaperone protein required for tumor growth; hypothesized that it prevents cellular death via circumventing stress due to cancer; associated with increased cell propagation in breast cancer
S100 Proteins	Ca^{2+} carriers involved in cell growth, differentiation, and cycle regulation; commonly thought to be potential breast cancer markers

Future work will include correlating antibody response to these specific antigens with vaccine efficacy and/or outcome.

We then addressed whether there are autoantibody signatures for early stage breast cancers. We purified IgGs from well-characterized serum samples from patients with newly diagnosed stage 1 (n=17) and 2 (n=23) invasive ductal carcinoma of the breast, and from age- and estrous cycle-matched healthy controls (n=15). The IgGs were labeled with fluorescent dyes and used as probes. Figure 3 (below) illustrates the reactivity in the cancer cases and normal controls.
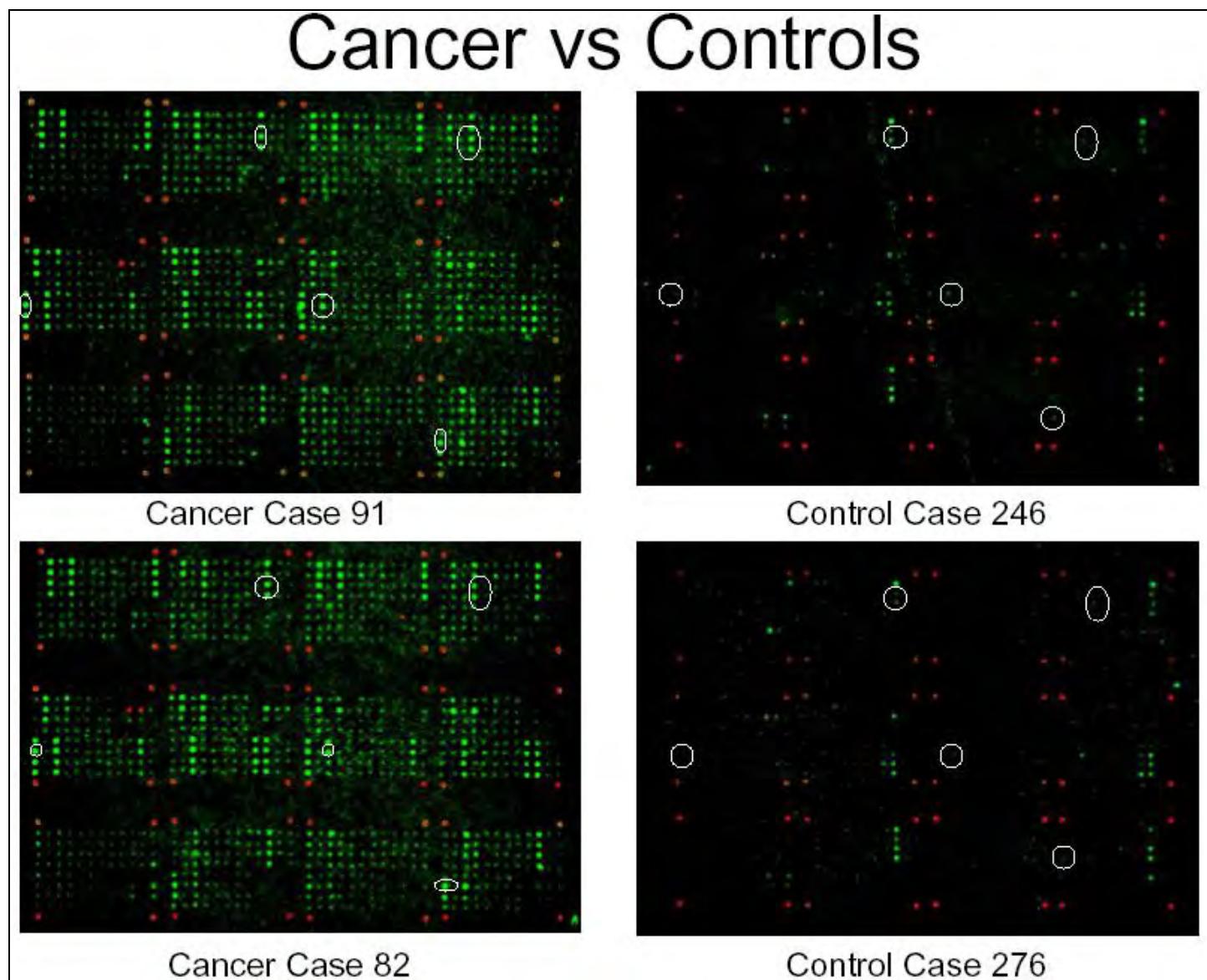
Figure 3: This figure illustrates representative antigen-autoantibody reactivity in 2 breast cancer cases and 2 normal controls. Each array was probed with Cy3 dye-labeled purified IgGs from either cases or controls. These images demonstrate that there is a high degree of similarity in autoantibody reactivity to the spotted fractions on the array in the cancer cases. Also note the limited autoantibody reactivity in the controls. Several fractions are circled to illustrate the strong antigen-autoantibody reactivity in the cancer cases but not in the normal controls.

To correct for intra- and inter-slide variations, a signal-to-noise ratio was computed from the difference of the intensity of each spot minus the background intensity of the spot divided by the standard deviation of the background intensity. Following normalization, Student T-tests were performed to identify the significant fractions with $p \leq 0.05$. The fluorescent intensities of these significant fractions were then used to perform 2-D hierarchical clustering and to construct a heat map that represents sample proximity. Figure 4 below is a heat map of 23 fractions with a statistical significance above 95% across all samples.
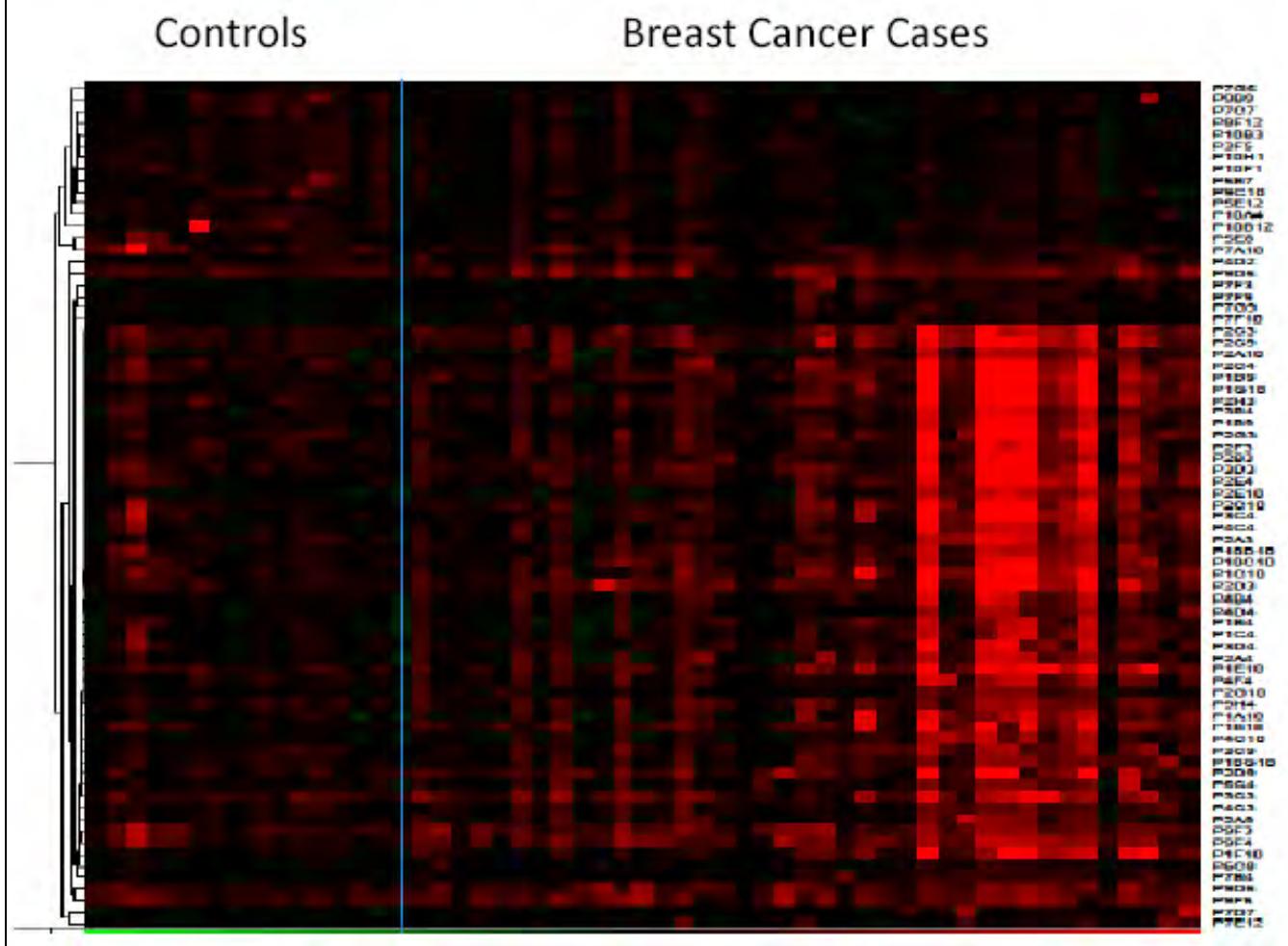
Figure 4: This heat map illustrates the spatial relationship between significant fractions of stage 1 and stage 2 breast cancer cases as well as controls. Significant fractions were selected following a Student T-test (p ≤ 0.05) and clustering was performed using a Euclidean similarity metric. The blue line separates the normal controls on the left from the cancer patients on the right. Increased fluorescent intensity (shown in red) for the breast cancer cases can be easily seen in several fractions.

Using these significant fractions, we constructed receiver operator characteristics (ROC) curves and area under the curve (AUC) calculations for the top 5 statistically significant fractions. The ROC curves are based on the fluorescent intensity values for each specific fraction from all patients with breast cancer and normal controls. After arranging the values from the highest intensity to the lowest intensity for a particular fraction, the fluorescent values were plotted on a sensitivity versus 1- specificity graph (sensitivity versus false positive) as previously described (4). From this curve, the AUC was calculated, which represents the predictive power of the autoantibody reaction for the fraction to distinguish between breast cancers and controls.

Figure 5 below illustrates our findings.

Cancer vs Normals
All Five fractions
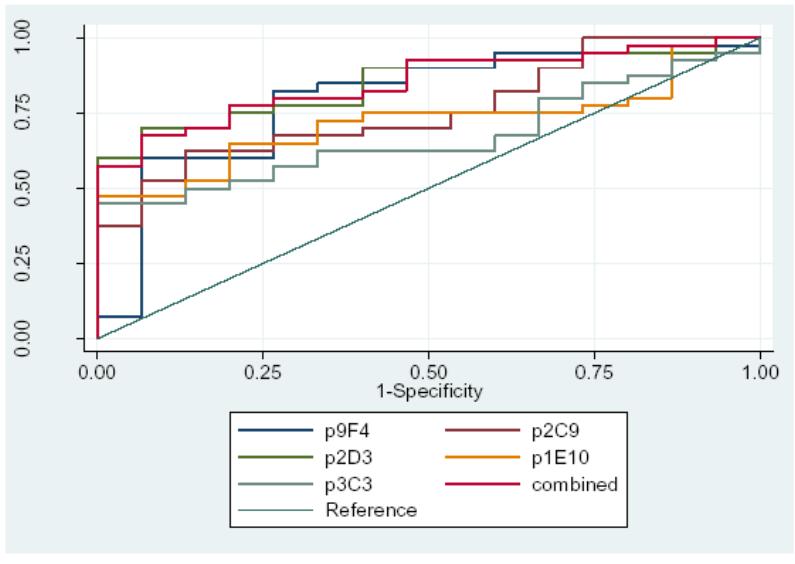
Figure 5: Figure 5 illustrates the ROC curves for the top 5 reactive fractions: p9F4, p2C9, p2D3, p1E10, and p3C3. A linear transformed model was used to obtain the ROC curve for the 5 fractions combined. The Y-axis is Sensitivity and the X-axis is 1 – Specificity (false positive). The AUC for p9F4 is 0.79; p2C9 is 0.75; p2D3 is 0.85; p1E10 is 0.72; and, p3C3 is 0.67. The AUC for all 5 fractions combined is 0.85. The reference line shows the expected plot at 50% sensitivity and 50% specificity.

We also separated our analysis be comparing stage 1 breast cancers versus normal controls and stage 2 breast cancers versus normal controls. ROC curves and AUC were performed. Figure 6 below illustrates our findings.
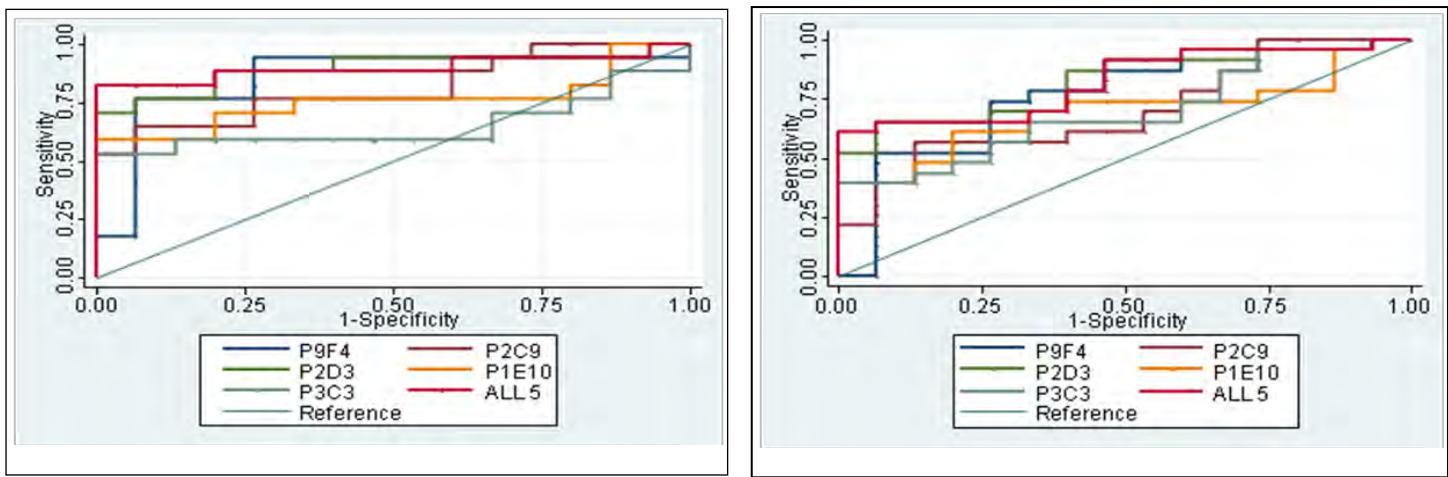
Stage 1 versus normal controls.

Stage 2 versus normal controls.

Figure 6: Figure 6 illustrates the ROC curves for the top 5 reactive fractions when we compared stage 1 breast cancers versus normal controls and stage 2 breast cancers versus normal controls. The top 5 reactive fractions (p9F4, p2C9, p2D3, p1E10, p3C3), when combined, gave an AUC of 0.89 for stage 1 breast cancers and 0.82 for stage 2 breast cancers. For stage 1 breast cancers, the AUC for p9F4 is 0.85; p2C9 is 0.80; p2D3 is 0.89; p1E10 is 0.75; and, p3C3 is 0.64. For stage 2 breast cancers, the AUC for p9F4 is 0.75; p2C9 is 0.69; p2D3 is 0.81; p1E10 is 0.69; and, p3C3 is 0.68. The reference line shows the expected plot at 50% sensitivity and 50% specificity.

The proteins in these fractions were identified by mass spectrometry. Table 2 lists some of the proteins identified in the 5 fractions.

Table 2: Some proteins that were identified by mass spectrometry in the top 5 reactive fractions.

Protein	Relation to Breast Cancer
NOP16	Nucleolar protein homolog which is upregulated in breast cancer; over expression often means poor survival rates
CFL1	Cofillin 1 is associated with stage 0, 1, and 2 breast cancers
Ki-67	Nuclear protein associated with, and may be necessary for, cell proliferation and is found in ductal breast cancers
STIP1	Stress induced phosphoprotein 1 causes cell proliferation in cancers

KEY RESEARCH ACCOMPLISHMENTS:

- Developed a whole proteome native fractionation microarray for breast cancer
- Identified potential antigen targets in an autologous cancer vaccine
- Identified potential autoantibody signatures for stage 1 and stage 2 breast cancers
- Demonstrated that the top 5 antigen containing fractions have an AUC of 0.89 for stage 1 breast cancers versus normal controls and an AUC of 0.82 for stage 2 breast cancers versus normal controls.
- Identified some of the putative autoantibody signatures by mass spectrometry

REPORTABLE OUTCOMES:

Our reportable outcomes are the key research accomplishments listed above. In addition, we have presented an abstract at the 2010 ASCO-NCI-EORTC Annual Meeting on Molecular Markers in Cancer in Hollywood, FL (October 17-10, 2010). This abstract was the recipient of a 2010 Merit Award. A more comprehensive abstract was presented at the 2011 Annual Meeting of the American Association for Clinical Chemistry in Atlanta, GA (July 24-28, 2011). The citation for the abstract is Clinical Chemistry 57(10), Supplement, A156, Abstract D-71, 2011.

CONCLUSION:

Our results demonstrate that we have successfully developed a whole proteome native antigen microarray platform to identify specific humoral immune responses. Our platform identified potential antigen targets in an autologous cancer vaccine as well as antigen containing fractions that appear to have significant clinical utility for early detection of breast cancers. As this was a pilot and feasibility study, additional work is needed to validate our findings in a larger and independent cohort of patients and controls.

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D-69

Comparison Of Architect I 2000 For Determination Of Scc With Imx

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Background: The SCC antigen, a tumor marker for squamous cell carcinoma, is already used for the diagnosis and follow-up of carcinoma of the cervix and the lungs. Serum concentrations of this marker correlate well with stage of disease, the presence or absence of risk factors, the effect of treatment, and the course of the disease.

Methods: The SCC concentration of 66 serum samples was determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 and MEIA (microparticle enzyme immnoassay) IMx Abbott diagnostic. All patients were hospitalized at Department of Gynecologic Oncology at the University Clinics Center of Sarajevo. The normal serum range of SCC lies between 0 and 2 ng/mL. The quality control, precision and accurancy of Architect i 2000 were assessed.

Results: The quality control was done using quality control serums for low (= 2.01ng/mL), medium (= 10.1ng/mL) and high (= 49.85ng/mL). We have got good precision with CV 2.70% to 2,98 %. We established that the main difference between Architect i 2000 and IMx was statistically significant at $p < 0.05$ according to Student t-test. Correlation coefficient was r = 0.990 and regresion line had a slope 1.8131and a y axis intercept of 0.1529. The good correlation with IMx SCC allows for efficient cross-over. The some samplers have higher concentration at Architect then at IMx because sensitive of Architect assay is high (< 0.1 ng/mL).

Conclusion: The CMIA Architect technology is an applicable method significant in diagnostic of SCC tumor marker.

D-70

Rapid detection of Influenza A and B viruses by real time reverse transcriptase loop-mediated isothermal amplification and multiplex real-time reverse transcriptase polymerase chain reaction

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Background: The lateral flow assay is the main diagnostic method for influenza viruses in point-of-care testing (POCT). However, false-negative results are noted in some cases of early-stage infection. Thus, this assay should be supported by high-sensitive genetic detection. However, real-time reverse transcriptase polymerase chain reaction (RT-PCR) and other RT-PCR methods are unavailable as POCT diagnostic methods, because of faults with equipments and the time required for general genetic detection. In this study, we focused on the use of real-time loop-mediated isothermal amplification (LAMP) and multiplex real-time RT-PCR in the rapid detection of Influenza A and B and other novel viruses. To achieve this aim, we designed corresponding primers.

Methods: Initially, individual primer sets targeting the common and/or few mutation regions of the nucleo protein (NP) gene and matrix (M) gene were designed to specifically detect Influenza A and B and novel influenza A (pandemic in 2009) viruses by real-time RT-LAMP and multiplex real-time RT-PCR. In addition, virus RNA were extracted from NATtrol virus sample (ZeptoMetrix Corp., Buffalo, NY) and 12 clinical samples by using the QIAamp Viral RNA Mini kit (QIAGEN, Dusseldorf, Germany). Next, the extracted RNA were analyzed for one-step NAT by using real-time RT-LAMP (Loopamp RT-160) and multiplex real-time RT-PCR (LightCyclerII, DX400, Roche, Basel, Switzerland), and were identified as Influenza A, B, or novel type virus. Moreover, the RNA extracted from the clinical samples of novel influenza A virus were sequenced by DNA sequencer CEQ 8800 (Beckman Coulter, Fullerton, CA) and identified. Finally, the results of the genetic assay were compared with those of the lateral flow assay.

Results and Discussion: In multiplex real-time RT-PCR, clinical samples and standard samples were identified using designed primers with a TaqMan probe. As a result, 6 clinical samples were identified as influenza A virus on the basis of the fluorescence intensity of the amplified cDNA. In LAMP, the clinical samples were identified using 6 primers designed for LAMP. The results reveal that influenza A and B viruses could be identified, and novel influenza virus could be detected in 3 of the 6 samples. The time required for detection after the extraction of RNA from the samples was 1 hour for both the methods. Thus, our real-time RT-PCR and LAMP assays are potentially useful in rapidly detecting influenza viruses such as A, B, and the novel type.

D-71

Development of a Whole Proteome Native Antigen Microarray Platform for Identifying Specific Humoral Immune Responses in Breast Cancer Sera

D. J. O'Rourke[1], D. A. DiJohnson[1], K. S. Anderson[2], B. C. S. Liu[3]. *[1]Brigham and Women's Hospital and Northeastern University, Boston, MA, [2]Dana-Farber Cancer Institute, Boston, MA, [3]Brigham and Women's Hospital, Boston, MA,*

Background: The humoral response of a cancer patient may allow earlier detection of cancer than current methods allow. If so, the serum autoantibody repertoire from cancer patients might be exploited for autoantibody profiling and aid in the serological diagnosis of cancer. In addition, specific humoral responses to cancer cells might occur following personalized vaccination. Our specific aims are to: 1) develop a whole proteome native antigen microarray platform that can be used to monitor humoral responses, and 2) test the hypothesis that global autoantibody profiling might identify relevant disease signatures.

Methods: Whole proteome native antigen microarrays were developed to identify potential antigen targets. Briefly, tumor antigens from breast cancer cells were separated into defined antigen fractions. This was accomplished by using 2-D liquid chromatography fractionation where the 1st dimension was separation by isoelectric points and the 2nd dimension was separation by hydrophobicity. Following the 2nd dimension, the fractions were arrayed onto nitrocellulose coated microscope slides. These spotted fractions contain proteins which have relevant post-translational modifications that are specific to the natural disease state.

To demonstrate the utility of the platform and to determine whether there are specific humoral responses to cancer cells following vaccination, we tested our platform on 7 patients who underwent tumor vaccination with autologous tumor cells engineered to express GM-CSF. Pre-vaccination and 2 month post-vaccination IgGs from the same patient were isolated and fluorescently labeled, and their reactivity to specific fractions on the microarray was compared. To address whether there are relevant autoantibody signatures for early stage breast cancer, we purified IgGs from well-characterized serum samples from patients with newly diagnosed stage 1 (n=17) and 2 (n=23) invasive ductal carcinoma of the breast, and from age- and estrous cycle-matched healthy controls (n=15). The IgGs were labeled with fluorescent dyes and used as probes.

Results: For patients who underwent tumor vaccination with autologous tumor cells, 54 fractions were determined to have a p-value of ≤ 0.05 when post-vaccination IgGs were compared to the patient's pre-vaccination IgGs. In addition to unique individual responses, 4 distinct fractions were found to have increased responses in over 50% of all post-vaccination IgGs. For sera from patients with stage 1 and 2 breast cancer, we identified 17 antigen containing fractions that were differentially reactive with the cancer sera ($P \leq 0.05$). Receiver operating characteristic curves were plotted for the top 5 reactive fractions and the area under the curve (AUC) was calculated. Our findings showed that when combined, the 5 reactive fractions have an AUC of 0.898 for stage 1 breast cancers versus controls (87% sensitivity at 80% specificity), and an AUC of 0.82 for stage 2 breast cancers. Identification by mass spectrometry found multiple cancer related antigens present in the fractions including NOP16, cofillin 1, and Ki-67.

Conclusion: Our results demonstrate that we have successfully developed a whole proteome native antigen microarray platform to identify specific humoral immune responses. Our platform identified potential antigen targets to cancer vaccines as well as antigen containing fractions that appear to have significant clinical utility for separating cancers from controls.

D-72

Rapid identification of *Escherichia coli* in clinical urine samples by fluorescence in situ hybridization

M. Wang[1], Y. Li[1], W. Li[2], X. Pan[1]. *[1]Department of Clinical Laboratory, Renmin Hospital of Wuhan University, Wuhan, China, [2]Department of Hematology, Renmin Hospital of Wuhan University, Wuhan, China,*

Background: *Escherichia coli* (*E. coli*) is the major pathogen in urine track infection (UTI). It can cause the asymptomatic bacterium urine and the acute nephropyelitis with noticeable symptom. Epidemiology reveals that UTI is common in female. In clinic, UTI can be diagnosed through bacteria culture of urine sample and the culture assay will take more than 48 hours. The aim of this study was to establish a rapid method for detecting and identifying *E. coli* in clinical urine samples.

Methods: Using fluorescent in situ hybridization (FISH) with 16S rRNA-targeted

Development of a Whole Proteome Native Antigen Microarray Platform for Identifying Specific Humoral Immune Responses in Breast Cancer Sera.

Dennis J. O'Rourke[1], Daniel A. DiJohnson[1], Karen S. Anderson[2], Brian C.-S. Liu[3]

[1]Brigham and Women's Hospital and Northeastern University, Boston, MA, [2]Dana-Farber Cancer Institute, Boston, MA,

[3]Brigham and Women's Hospital, Boston, MA
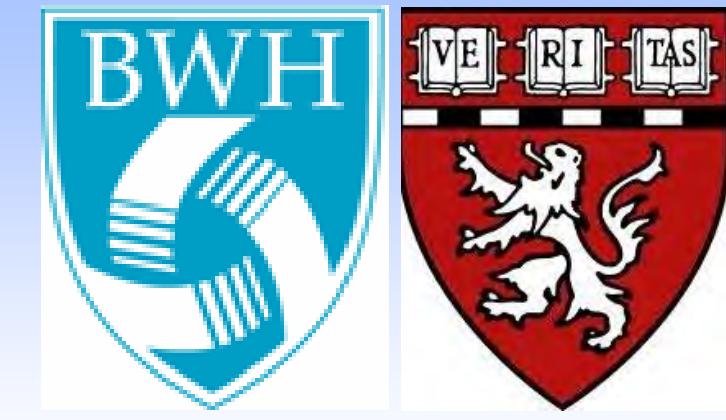
Introduction

Breast cancer is the most common cancer among women in the United States, afflicting over 200,000 per year. Also, excluding lung cancer, it is responsible for the most cancer related deaths among females each year. There are various treatment modalities, however, most still have devastating side effects and early detection is still limited. The humoral immune response of a cancer patient may allow earlier detection of cancer than current methods. If so, the serum autoantibody repertoire from cancer patients might be exploited for autoantibody profiling and aid in the serological diagnosis of cancer. In addition, specific humoral immune responses to cancer cells might occur following personalized vaccination.

Therefore, our specific aims of this study are to: 1) develop a whole proteome native antigen microarray platform that can be used to monitor humoral immune responses, and 2) test the hypothesis that global autoantibody profiling might identify relevant disease signatures.

Methods

To construct the whole proteome native antigen microarray platform we fractionated tumor lysate collected from HCC38 breast cancer cells. The lysate was fractionated using a two dimensional approach where the first fractionation was by isoelectric point and the second by hydrophobicity. Following fractionation, each fraction was arrayed onto nitrocellulose coated microscope slides. These fractions contain native antigens with proper post-translation modifications which may be specific to breast cancer

### Figure #1: Overview of the Microarray Creation Process
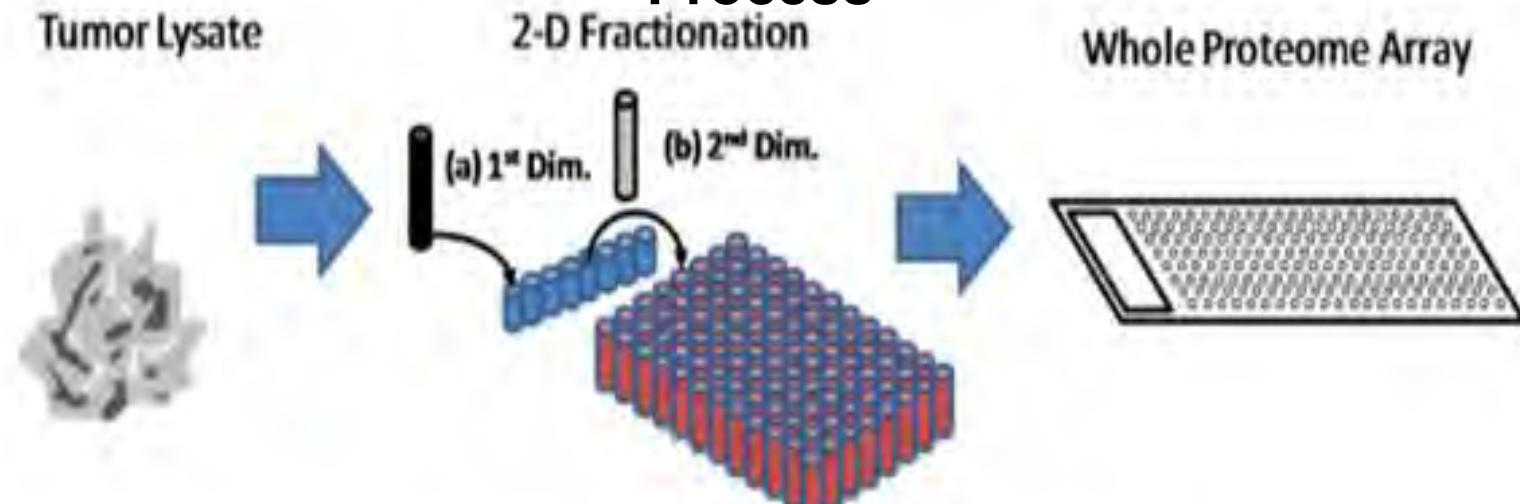
Figure #1 shows the general creation of the antigen microarray slide. Tumor cells are lysed and then fractionated by 2D liquid chromatography. The fractions are put into ten 96-well plates and aliquots of each are then arrayed onto the slide.

Vaccination Experiment

Under IRB approval, patients with late stage breast cancer underwent tumor vaccination with autologous tumor cells engineered to express granulocyte macrophage-colony stimulating factor. The tumor cells were then lethally irradiated and subcutaneously injected back into the donor patient. Patients were inoculated on days 1, 8, 15, 29, and then every two weeks until the vaccine supply ran out. We obtained both pre- and post-vaccination serum samples from each patient.

IgG from the collected samples was then purified and dye labeled. Pre-vaccination IgG was dye labeled with a red fluorescing Cy5 dye and post-vaccination IgG with a green fluorescing Cy3 dye. The dye labeled samples were then incubated on the whole proteome array, allowing for proper binding to the printed antigens. The slides were scanned for fluorescent intensity, quantified and analyzed using bioinformatics software.

### Figure #2: Overview of the Antigen Microarray Platform Protocol
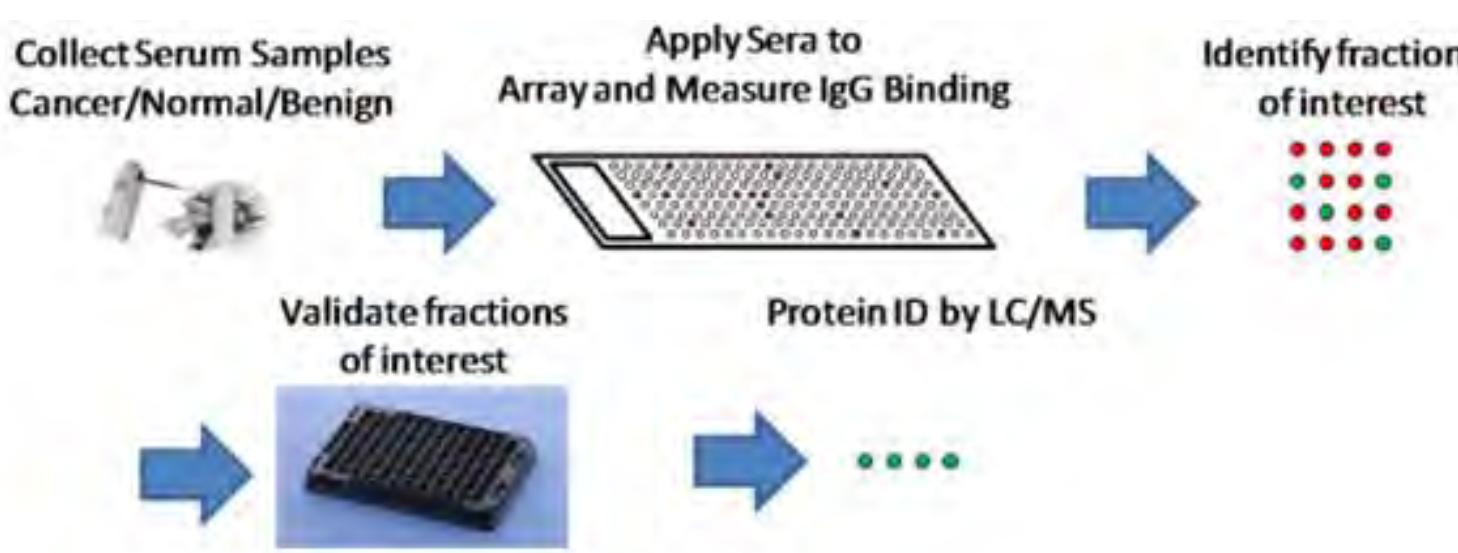
Figure #2 shows patient serum was collected pre- and post-vaccination and labeled with fluorescent dyes. The two sera samples from each patient were then incubated with the microarray slide, resulting in spots fluorescing either green or red. In one dye orientation, the green fractions suggest increased antibody reactivity due to the vaccine and therefore would be the fractions validated by mass spectrometry.

Early Detection Experiments

The same protocol to isolate and dye label IgG from patient samples was used as described above. However in these experiments we compared either stage 1 breast cancer patients or stage 2 breast cancer patients against healthy, age- and estrous cycle-matched controls. Also, all samples were dye labeled with the same dye and individual samples were incubated on their own array.

Results

For patients who underwent tumor vaccination with autologous tumor cells, 54 fractions were determined to have a p-value of ≤0.05 when post-vaccination IgG was compared to pre-vaccination IgG. In fact, 4 of these fractions were found to have increased responses in over 50% of all post-vaccination IgG. The proteins in these fractions were then identified using mass spectrometry.

Figure #3: Antigen Microarray Image with Cy3 and Cy5 Fluorescence
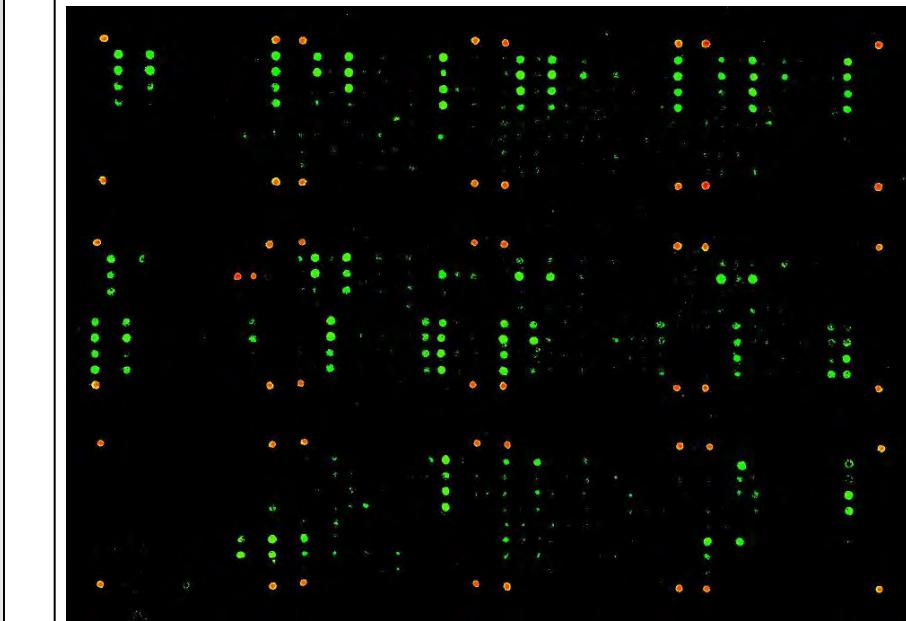
Figure #3 is an image of a microarray slide scanned for fluorescence after incubating with Cy3 labeled (green) post-vaccination serum and Cy5 labeled (red) pre-vaccination serum.

Table 1: Proteins found in the fractions of interest through mass spectrometry

Protein	Relation to Breast Cancer
Mammaglobin-B	A breast cancer diagnostic marker; highly similar to mammaglobin-A which is over expressed in 23% of primary breast cancer tumors; limited to the epithelial tissue of the mammary glands
Lactotransferrin	Iron transporting glycoprotein with serine protease activity, both of which contribute to its antimicrobial properties; thought to inhibit tumor growth by halting the cell cycle at the G1 to S checkpoint
MUC5B	O-glycoprotein up-regulated in epithelial breast cancer tissue; absent from normal tissue
HSP70	Chaperone protein required for tumor growth; hypothesized that it prevents cellular death via circumventing stress due to cancer; associated with increased cell propagation in cancer
S100 Proteins	Ca²⁺ carriers involved in cell growth, differentiation, and cycle regulation; commonly thought to be potential breast cancer markers

Likewise when comparing stage 1 breast cancer or stage 2 against healthy controls, we were able to identify 17 fractions that were differentially reactive with the cancer sera corresponding to a p-value of ≤0.05. The 5 most reactive fractions were identified, ROC curves were produced, and AUC values were calculated.

Figure #4: ROC curves for stage 1 breast cancer versus normals (left) and stage 2 breast cancer versus normals (right)
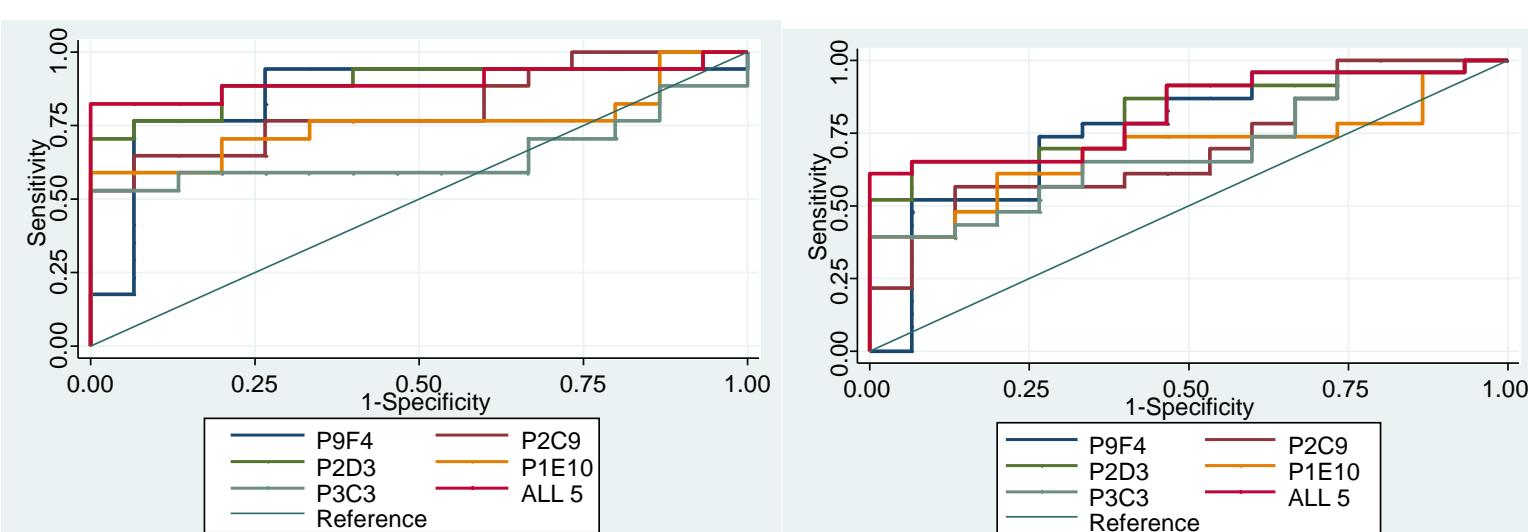
When stage 1 breast cancer was compared with normals, the 5 fractions of interest, when combined, showed an area under the curve of 0.89. Likewise, when stage 2 breast cancer was compared with normals, the five fractions of interest, when combined, showed an area under the curve of 0.82. The proteins in these fractions of interest were identified using mass spectrometry. Below are some of the breast cancer related proteins found in the fractions.

Protein	Relation to Breast Cancer
NOP16	Nucleolar protein homolog which is upregulated in breast cancer; over expression often means poor survival rates
CFL1	Cofillin 1 is associated with stage 0, 1, and 2 breast cancers
Ki-67	Nuclear protein associated with, and may be necessary for, cell proliferation and is found in ductal breast cancers
STIP1	Stress induced phosphoprotein 1 causes cell proliferation in cancers

Conclusion

Our results demonstrate that we have successfully developed a whole proteome native antigen microarray platform to identify specific humoral immune responses. Our platform identified potential antigen targets to cancer vaccines as well as antigen containing fractions that appear to have significant clinical utility for early detection of breast cancer.